

AMENDMENT TO THE SPECIFICATION

Please replace the amended paragraph found on page 6, line 20 - page 7, line 30 with the following paragraph.

Media used to culture seedlings to obtain explant material was designed to minimize browning of the roots (browning adversely effects the ability of explants to grow in culture and form callus), and to promote overall vigorous root growth. In one embodiment, MET (multi-effect triazole, a chemical agent used in agriculture to promote root growth) is used in the seedling culture medium. In a preferred embodiment MET (~~multi-effect triazole, a chemical agent used in agriculture to promote root growth~~) and NAA (α naphthalene acetic acid) are used together in the seedling culture medium to reduce the proportion of browned roots and increase callus initiation rate. MET is preferably used in concentrations ranging from about 0.05 mg/l to about 0.2 mg/l, most preferably about 0.1 mg/l. NAA is preferably used in concentrations ranging from about 0.01 mg/l to about 0.2 mg/l, most preferably about 0.05 mg/l. MET and NAA are also preferably used in the medium used to root transgenic seedlings regenerated from callus, in amounts similar to those described for the seedling culture medium. In a preferred embodiment of the callus-forming medium vitamin B₅, 2,4-D ((2,4-dichlorophenoxy)acetic acid, MgCl₂ and glucose are used, preferably about 0.05 mg/l to about 0.15 mg/l 2,4-D, about 0.4 mg/l to about 1.2 mg/l MgCl₂, and about 1% to about 5% glucose, most preferably about 0.1 mg/L 2,4-D, 0.8 mg/L MgCl₂ and 3% glucose. In an alternate preferred embodiment of the callus-forming medium myo-inositol, vitamin B₁, and dimethylallyl(amino)purine are used, a, preferably about 50 mg/l to about 150 mg/l myo-inositol, about 1 mg/l to about 10 mg/l vitamin B₁, and about 0.1 mg/l to about 7.5 mg/l dimethylallyl(amino)purine, most preferably about 100 mg/l myo-inositol, about 0.4 mg/l vitamin B₁ and about 5 mg/l dimethylallyl(amino)purine. The same media used for callus induction can also be used during selection with antibiotics -- for example with 300-400 mg/L cefotaxime or 15-30 mg/L kanamycin. The presence of high concentrations (preferably about 1900 mg/l to about 5700

Serial No. 09/936,173
Amendment dated 9 August 2007
Reply to Office Action dated 9 February 2007

mg/l, most preferably about 3800 mg/L) of nitrates was crucial for the observed effectiveness of the differentiation medium. With the fibrous roots as explants, although the rate of callus-induction was lower compared with hypocotyl and cotyledon, a higher rate of transformation was achieved.